



6-22-04

1636  
IPW

PTO/SB/21 (02-04)

Approved for use through 07/31/2006. OMB 0651-0031  
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

## TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission

11

Application Number 10/068,664

Filing Date 02/06/2002

First Named Inventor Chuan Li

Art Unit 1636

Examiner Name James S. Ketter

Attorney Docket Number

### ENCLOSURES (Check all that apply)

- |  |   |   |
|--|---|---|
| <input type="checkbox"/> Fee Transmittal Form                                | <input type="checkbox"/> Drawing(s)   | <input type="checkbox"/> After Allowance communication to Technology Center (TC)        |
| <input type="checkbox"/> Fee Attached  | <input type="checkbox"/> Licensing-related Papers                                       | <input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences     |
| <input checked="" type="checkbox"/> Amendment/Reply                          | <input type="checkbox"/> Petition   | <input type="checkbox"/> Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) |
| <input type="checkbox"/> After Final   | <input type="checkbox"/> Petition to Convert to a Provisional Application               | <input type="checkbox"/> Proprietary Information  |
| <input type="checkbox"/> Affidavits/declaration(s)                           | <input type="checkbox"/> Power of Attorney, Revocation Change of Correspondence Address | <input type="checkbox"/> Status Letter  |
| <input type="checkbox"/> Extension of Time Request                           | <input type="checkbox"/> Terminal Disclaimer  | <input type="checkbox"/> Other Enclosure(s) (please identify below):                    |
| <input type="checkbox"/> Express Abandonment Request                         | <input type="checkbox"/> Request for Refund   |   |
| <input type="checkbox"/> Information Disclosure Statement                    | <input type="checkbox"/> CD, Number of CD(s) _____                                      |   |
| <input type="checkbox"/> Certified Copy of Priority Document(s)              | <b>Remarks</b><br>Transmittal Form (1 page) and Amendments (10 pages)                   |   |
| <input type="checkbox"/> Response to Missing Parts/ Incomplete Application   |   |   |
| <input type="checkbox"/> Response to Missing Parts under 37 CFR 1.52 or 1.53 |   |   |

### SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual name

Chuan Li

Signature

*Chuan Li*

Date

June 21, 2004

### CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below.

Typed or printed name

Chuan Li

Signature

*Chuan Li*

Date

6/21/04

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.



Amendments to DE NOVO SYNTHESIZED PLASMID, METHODS OF MAKING  
AND USE THEREOF

Application/Control Number: 10/068,664

Art Unit: 1636

**a.) Introductory Comments**

1. In ABSTRACT, the title “De novo synthesized plasmid, methods of making and use thereof” is deleted.
2. In claim 1, the phrase “without referring an existing plasmid as a template” is added after phrase “A de novo synthesized plasmid” to make the scope of the claim clear.

In part (a) of claim 1, the term “relevant to” is changed to “causing” according to suggestion.

In part (b) of claim 1, the term “relevant to” is changed to “causing” according to suggestion.

In part (b) of claim 1, the term “the” before “selection of a plasmid” is deleted.

In claim 2, the phrase “the plasmid previously obtained from natural sources” is changed to “an existing plasmid previously obtained from natural sources” to address antecedent basis.

In claim 2, the term “modified” is changed to “synthesized” to make the claim clear.

In claim 2, the phrase “as a template” is added to the end of the sentence to make the claim specific.

In claim 3, the phrase “the plasmid previously obtained from recombinant sources” is changed to “an existing plasmid previously obtained from recombinant sources” to address antecedent basis.

In claim 3, the term “modified” is changed to “synthesized” to make the claim clear.

In claim 3, the phrase “as a template” is added to the end of the sentence to make the claim specific.

In claim 4, the phrase “wherein the replication origin allows the autonomous plasmid replication in a host cell” is changed to “wherein the replication origin allowing the autonomous plasmid replication in a host cell is from natural or recombinant sources” to make the claim specific. This change also makes claims 2 and 3 clear that the replication origin of the plasmid is from natural or recombinant sources whereas the plasmid is de novo synthesized.

In claim 5, the phrase “wherein the selection marker gene encodes a product indicative of plasmid maintenance in a host cell” is changed to “wherein the selection marker gene encoding a product indicative of plasmid maintenance in a host cell is from natural or recombinant sources” to make the claim specific. This change also makes claims 2 and 3 clear that the selection marker of the plasmid is from natural or recombinant sources whereas the plasmid is de novo synthesized.

3. The applicant believes the claimed invention is not anticipated by Stemmer et al. (cited of record in the information disclosure Statement filed 12 January 2004, now numbered as reference 5) for those skilled in the art at the time the invention was made. The reasons are following:
  - 1). The processes of making the plasmids are different between the teaching of Stemmer et al. and the claimed invention. Stemmer et al. makes the plasmid pUC182Sfi using 134 oligos (page 50, left hand column, last paragraph and right hand column, first paragraph, page 51, first paragraph, and page 51, Fig. 3). They performed three-stage PCRs. Their first PCR has 19 reactions (Fig. 3B) and their second PCR has 11 reactions (Fig. 3C). After third PCR, they need perform restriction digestion and ligation to transform bacteria. Most importantly, they use an existing plasmid as a template. The claimed invention makes the plasmid p4T using only 4 oligos (44, 32, 32, and 32 nucleotides in length respectively, Example 1 of the applied invention). One-stage PCR with two reactions are performed. The PCR products are subjected to a brief Exonuclease III digestion (30 seconds reaction, Li et al. Nucleic Acid Res. 25: 4165-4166 (1997)) and then transform the bacteria without further restriction digestion or ligation. Most importantly, p4T is de novo synthesized without using an existing plasmid as a template. The process described in the claimed

invention has fewer steps and it is significantly simpler and much faster than the process used by Stemmer et al.

2). The resulting plasmids made by Stemmer et al. and by the claimed invention are different. Stemmer et al. synthesized an existing plasmid pUC182Sfi (Stemmer et al. Nature 370, 389-391 (1994)) with known properties using pUC182Sfi as a template. pUC182Sfi can also be readily synthesized by modifying pUC18 using site-directed mutagenesis by those skilled in the art at time the paper was published. In contrast, p4T is a de novo synthesized novel plasmid with unknown properties. It is relatively difficult to make p4T by modifying existing plasmid pACYC177 with current recombinant DNA technology. On the other hand, those skilled in the art would not have the incentive to make the novel plasmid p4T with unpredictable properties. The replication origin of p4T is generated from low copy number plasmid pACYC177; its predicted copy number should be lower than plasmids synthesized from pBR322 and significant lower than the plasmids synthesized from pUC19. However the observed copy number of p4T is higher than or comparable with the plasmids synthesized from pUC19 origin (p3A, Fig. 2) and significant higher than those synthesized from pBR322 origin (p1A, Fig. 2). This unexpected result, which is contradictory to relevant teachings in molecular biology, is not anticipated by Stemmer's teaching or any other prior arts.

3). The uses of the plasmids made by Stemmer et al. and by the claimed invention are different. p182Sfi made by Stemmer et al. (Stemmer et al. Gene 164, 49-53 (1995)) has same uses of the same plasmid made previously (Stemmer et al. Nature 370, 389-391 (1994)). It has similar properties and uses as that of pUC18. In contrast, the replication origin of p4T is synthesized from pACYC177 and its selection marker gene is synthesized from pBR322. The novel plasmid p4T is de novo synthesized without referring either pACYC177, pBR322 or any other plasmids as a template. p4T has higher copy number than pACYC177 and stronger tetracycline resistance than pBR322 (unpublished results). Therefore it has different uses than pACYC177 and pBR322. For example, p4T can be used in the applications that require high copy number and at same time with the same replication origin as pACYC177 while

neither pBR322 nor pACYC177 can be used. Stemmer et al. does not teach the claimed invention.

In conclusion, the disclosed invention uses different process to make plasmid. The de novo synthesized plasmid has novel sequences and novel, sometimes unexpected properties. It is useful in various biomedical applications. It is neither anticipated nor obvious over any prior arts or combination of them. Therefore Claims 1-5 are patentable.